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Persistent and recurrent nasopharyngeal carcinoma

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Introduction & outline of the thesis

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Introduction

Nasopharyngeal carcinoma (NPC) is a rare disease worldwide, but in certain regions the prevalence is high. In the Netherlands, 40 to 80 new cases are diagnosed each year (1). The highest incidence of NPC has been found among the Cantonese people living in the Guangdong province in China, with an incidence up to 27 per 100,000 (2). The worldwide top 3 countries with the highest-incidence are Malaysia, Singapore and Indonesia (3). In North and East Africa the incidence ratios are as high as 3.2 per 100,000 (Algeria). In Europe and North America NPC is rarely observed, with an incidence below 0.5 per 100,000. However, in South-East Europe and in Caribbean countries, higher incidences are found; up to 1.0-1.9 per 100,000 (Fig. 1a). Taken together, more than 90% of the patients diagnosed with NPC reside in developing countries (Fig. 1b) (3).

Already for decades, the unique geographic distribution of NPC's etiology has been a major topic for studies. Across all populations, men are more affected than woman, with a ratio of 2.5:1. Age-specific incidence rates differ among regions. In the high-incidence countries, the peak incidence is in the 40-60 age group and in the low-incidence regions the peak is in the 60-70 age group (4, 5). Early life exposure to carcinogens in the high-risk regions may be an explanation for this difference. Epstein-Barr virus (EBV) infection has the strongest proven correlation to NPC. Other environmental risk factors associated with NPC are salted fish (Cantonese style) and other salt-preserved foods, alcohol consumption and herbal medicines. More risk factors have been found, but these remain to be confirmed (betel nuts, domestic wood cooking, occupational wood exposure, incense, and formaldehyde) (5-7). Consumption of fresh fruit and vegetables may give protection against NPC, due to the content of anti-oxidants. Tobacco smoking is studied intensively as a risk factor for cancer, and thus also for NPC. The increased risk is certain for keratinizing NPC, but for non-keratinizing NPC study results are inconsistent. This inconsistency seems to be caused by different risk factors among ethnicities (5-8).

Besides environmental factors, genetic predisposition plays a role in the development of NPC. This was proven by the different incidence rates among ethnicities living in the same region/environment and by the findings that when 'ethnicities at risk' moved to low-risk areas the increased risk remained (2, 9). Moreover, NPC is more frequently diagnosed among people with a family history of NPC. This correlation is stronger in high-incidence regions compared to low-incidence regions (5, 10). Currently, many studies focus on genetic susceptibility loci for NPC. One of the

current hypotheses is that susceptibility for NPC is based on a deficient clearance of EBV. Several specific Human leukocyte antigen (HLA) regions, but also many other gene polymorphisms and mutations have been identified (7, 8, 11, 12). Later in this chapter, more attention is drawn to the relationship between EBV and NPC.

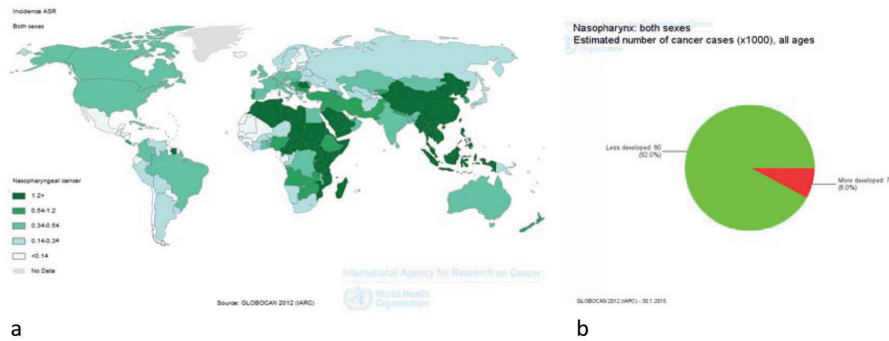


Figure 1. (a). Worldwide incidence (ASR) of NPC. (b) NPC incidence distribution over more and less developed countries. Source: Globocan 2012 (IARC) (3)

Anatomy



Figure 2. Anatomy of the nasopharynx. Source: Hirschfeld

The nasopharynx is a tubular space, hidden in the center of the head. It forms the transition from nasal cavity to the oropharynx. The roof of the nasopharynx is defined by the skull base, which contains the sphenoid sinus and the cavernous sinuses. In the lateral walls, the orifices of the Eustachian tube are found and medially to these the fossa of Rosenmüller. Anteriorly, the nasal cavities can be found and the floor

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consists of the soft palate. The best way to visualize the nasopharyngeal space is by endoscopic examination. The roof of the nasopharynx and the fossa of Rosenmüller are typical locations of origin for NPC (Fig. 2).

Presentation

The local mass in the nasopharynx can give symptoms like; epistaxis, nasal discharge, nasal obstruction, or unilateral dysfunction of the Eustachian tubes, leading to tinnitus, otitis media or impaired hearing. Early on, these complains can mimic an ordinary upper airway infection. Due to further local progression of NPC in the skull base or intracranial region with cranial nerve involvement, headache, trismus, dysphagia, dysarthria, diplopia, facial pain or numbness can be observed. The most affected nerves are n. V, VI, III and XII. Nevertheless, the most common presenting symptom for NPC is a painless, enlarged upper neck mass. Classically, this is located in level 2, but any cervical lymph node area can be involved. Due to the non-specific complaints of the local mass, the majority of the patients is diagnosed only at an advanced stage of the disease (13, 14).

Pathology

Based on histology, the World Health Organization (WHO) distinguished NPC in well-differentiated keratinizing squamous cell carcinoma (WHO 1) and non-keratinizing carcinoma (WHO 2), and undifferentiated carcinoma (WHO 3). The latter two are strongly related to EBV. Undifferentiated non-keratinizing NPC coincides for almost 100% with EBV and is the most frequently observed type of NPC, both in endemic (>95%) and non-endemic (44-63%) regions (1, 15).

Staging

The most widely used classification system for NPC is the Union for International Cancer Control (UICC) and the American Joint Committee staging system on Cancer (AJCC). The latest version dates from 2010 and is the 7th edition (table 1). In comparison to the 6th edition a few changes have been made (16). For local tumor staging, involvement into the nasal cavity or oropharynx showed similar prognosis as T1, therefore T2a is T1 in the 7th edition and parapharyngeal extension T2 (previously T2b). For nodal staging, the role of retropharyngeal lymph node is clarified in the 7th edition. Uni- or bilateral retropharyngeal lymph node invasion is now staged as N1.

Table 1. TNM-staging according to UICC and AJCC 7th edition

Tumor in the nasopharynx	
T1:	Nasopharynx, oropharynx, or nasal fossa
T2:	Parapharyngeal extension
T3:	Bony structure, paranasal sinuses
T4:	Intracranial extension, cranial nerve, hypopharynx, orbit, infratemporal fossa (masticatory space)
Regional lymph nodes	
N0:	None
N1:	Unilateral cervical, uni/bi-lateral retropharyngeal, <6 cm, above supraclavicular fossa
N2:	Bilateral cervical node, <6 cm, above supraclavicular fossa
N3a:	Nodes >6 cm
N3b:	Nodes in the supraclavicular fossa
Distant metastasis	
M0:	No distant metastasis
M1:	Distant metastasis
Stage grouping	
Stage I:	T1 N0 M0
Stage II:	T1 N1 M0 T2 N0-1 M0
Stage III:	T1-2 N2 M0 T3 N0-2 M0
Stage IVA:	T4 N0-2 M0
Stage IVB:	Any T N3 M0
Stage IVC:	Any T Any N M1

Treatment

NPC is responsive to both radiotherapy and chemotherapy. In the past decades many studies have been performed to optimize the treatment for NPC, and have done so with success. In the early days, treatment for NPC consisted of radiotherapy alone. The median 5-year survival was 24-62%, with a high loco-regional failure rate of 40-80% and a high incidence of distant metastasis (15-50%) (17-19). Through the introduction of chemotherapy these statistics have improved considerably.

In 1998, 'The Intergroup study 0099' was the first of its kind that proved the benefit of chemotherapy on progression free survival and overall survival (20). Since then, the addition of chemotherapy has become the standard for treatment of advanced NPC. Multiple chemotherapeutics have been studied. Effective single agents are methotrexate, bleomycin, doxorubicin, adriamycin, carboplatin, and taxanes

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(17, 18). Combined therapies are more effective than single agent therapy, and in particular cisplatin-based regimens are effective. Chemotherapy can be given in a neo-adjuvant (induction chemotherapy followed by radiation), concurrent (chemotherapy and radiation concomitantly), or adjuvant (chemotherapy after radiotherapy) schedule. Also, combinations of these are possible. All combinations have been studied, with varying results. The meta-analysis by Langendijk *et al.* showed an absolute increase in overall survival when chemotherapy was added to radiotherapy (21). Subgroup analysis showed that this effect only remained significant when concurrent chemotherapy was used. The absolute benefit in survival was 20% in 5 years. To date the National Comprehensive Cancer Network recommends concurrent chemo-radiotherapy with cisplatin-based regimens for stage II and higher (22).

The actual benefit of additional adjuvant and/or neo-adjuvant chemotherapy is still up for discussion. Besides the uncertainty of better tumor control, the addition of another course of chemotherapy may be too toxic. In 'The Intergroup study 0099', adjuvant chemotherapy was added to concurrent therapy, which resulted in a decrease in compliance rate of 55%, due to additional toxicity (23).

Besides chemotherapy, radiotherapy treatment has also gone through some changes in the past years. In the beginning, radiation was only possible in 2 dimensions. Later this was changed into 3-dimensional radiotherapy. Nowadays multi-direction radiation planning is the standard of treatment in most well equipped hospitals. The advantage of intensity-modulated radiotherapy (IMRT) above 2D and 3D radiation is a higher dose on the targeted tumor, while minimizing the dose to the surrounding structures, aiming for better tumor control and less side effects. In particular, the salivary glands are spared by IMRT treatment (24, 25). A disadvantage of IMRT is the risk on geographic misses. Peng *et al.* (2012) (n=616) found a benefit in 5-year survival in NPC in favor of IMRT; 80% and 67% for IMRT and 2D respectively. Local control and regional control were not significantly different. Radiation-induced toxicities were lower in the IMRT group compared to those in the 2D radiotherapy group (26). In Yogyakarta, the 3D technique is available, nevertheless due to the longer radiation time and the long waiting lists, the 2D technique remains the standard treatment for most patients.

Management of loco-regional failures

Close monitoring of patients post-treatment is important since early detection of recurrent disease significantly affects the chance on survival. Local failure occurs in 10% to 30% of patients and regional failures occur in less than 5% (13, 15, 27-29). For regional failures, a neck dissection is indicated, this gives a 5-year control rate of 66% (13, 15). Treatment of local failures is challenging, due to the difficulty of reaching the hidden nasopharynx and the proximity of critical structures. Treatment options include surgery (endoscopic or open), external beam re-irradiation (conventional or IMRT), brachytherapy, radiosurgery, chemotherapy and photodynamic therapy (PDT). No consensus has been reached for the best treatment option.

Loco-regional failure is a major problem in Indonesia; the health care management is less developed and the availability of radiotherapy units (in particular IMRT), equipment and surgeons with experience to perform the complicated nasopharyngectomy is limited. Since the majority of the NPC patients are diagnosed in less developed regions, like Indonesia, it is likely that many developing countries also have to cope with these problems (Fig. 1b) (3). Alternative treatments, like PDT could be a solution in these regions.

Photodynamic therapy

PDT is based on administration of a photosensitive drug, and illumination of the tumor by laser with a wavelength of 652 nm. This results in tumor destruction (30-33). Several clinical trials with first generation haematoporphorin-derived photosensitizers (HpD or Photofrin) have shown that PDT is effective in destroying NPC. Good local tumor control and complete responses were found in the majority of patients with limited recurrent or persistent disease, while achieving long-term palliation in cases with extensive recurrence (34-37). Although these results were encouraging, PDT for NPC has not yet been considered a break through. Drawbacks in studies that questioned PDT were the light delivery and the selection of the photosensitizer. Complete illumination of the tumor is difficult, due to the location of the nasopharynx, which is deeply hidden in the head, and the closely surrounding vulnerable structures, like the soft palate. Secondly, the used photosensitizers (first generation) have a depth penetration of a maximum of 5 mm and a light hypersensitivity of several months.

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To overcome these challenges, a special nasopharyngeal applicator was developed. This applicator allows one-stage illumination of the entire nasopharynx (Fig. 3) (38). Also, a new (second-generation) photosensitizer was used. Temoporfin (Foscan®), has a depth penetration of 1 cm and light hypersensitivity of only a few weeks. Research in NPC cell lines confirmed a much better efficiency than HpD (39). Therefore, Temoporfin in combination with the applicator has high potential to treat NPC effectively. In a phase I study, in Yogyakarta, this combination was used for the treatment of 22 patients with recurrent and persistent NPC restricted to the nasopharynx. The therapy showed to be relatively simple to perform and well tolerable under local anesthesia. Also, clinical benefit was seen (31). In this thesis the continuation of this study is presented (chapter 4).

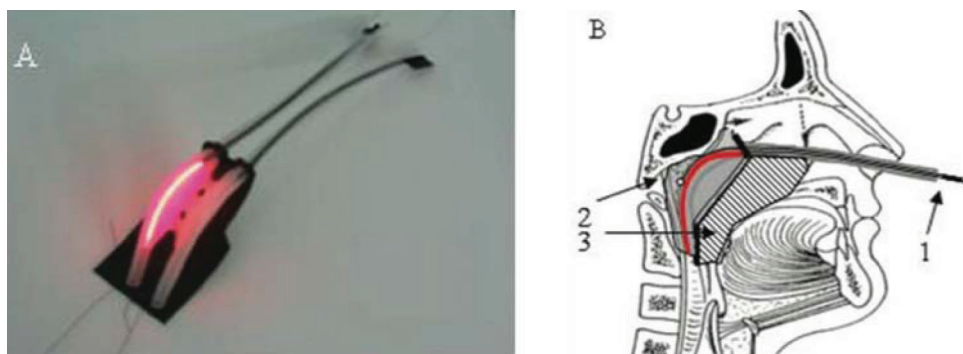


Figure 3. Nasopharyngeal applicator for photodynamic therapy;

A applicator; B schematic view of positioning and illumination. 1. Cylindrical diffuser in shielding tube. 2. Target area. 3. Soft pallet is shielded. (source Nyst et al. 2007 (38))

Management of distant metastases

In the Netherlands, 5% of the newly diagnosed NPC patients have distant metastasis (1). In Indonesia 14% of the newly diagnosed NPC patients have distant metastasis at diagnosis (40, 41). In case of distant metastasis at diagnosis, the median survival is 10 to 26 months, depending on the dissemination pattern and choice of palliative treatment (42). When distant metastases occur after platinum-based regimens, palliative treatment with gemcitabine, capecitabine or docetaxel, results in a median survival of 9.5-15 months (43). There is great interest in improving survival for these patients by novel strategies.

Novel strategies in NPC

To date, the molecular biology of cancer and its effects on the immune system has garnered much attention in an attempt to find novel treatment modalities. Also in NPC this is a hot topic (22, 44-46). The main approaches of novel molecular therapies in NPC are; targeting of signal transduction and angiogenesis, modulation of gene expression, and cancer immunotherapy. Although, a firm number of pre-clinical studies have booked interesting results, large clinical studies are limited (46).

A few trials with epidermal growth factor receptor inhibitors and angiogenesis inhibitors showed clinical benefit, however, serious adverse events occurred (10). Immunotherapy can involve a broad range of strategies. Roughly the goals can be categorized in; increased tumor antigenicity (making the tumor cells better recognizable for the immune system), increased immunological response (for a stronger and longer lasting immune response), and a decreased immune escape of tumor cells. The presence of EBV in NPC makes it a potential target for immunotherapy. The strategy for a stronger immune reaction is the administration of, or expansion of, EBV-specific cytotoxic T lymphocytes (CTL) (45). Expansion of the EBV- specific CTL in NPC is managed, but the actual clinical benefit in terms of tumor response needs to be improved. Common hurdles include that NPC tumor cells are not recognizable for the CTL and that CTL might not reach its target destination (nasopharynx or place of metastatic disease) in an active state (10, 45). Currently, several clinical trials are being performed to attest improvement strategies of EBV specific CTL therapy in NPC (47). In immune-checkpoint blockades, one of the promising targets is the Programmed cell death pathway. Programmed death-1 (PD-1) is a cell-surface receptor that is expressed on lymphocytes. After it binds with PD-1 ligands (PD-L1) located on the cell surface of the tumor, it inhibits lymphocyte proliferation and effector functions. It is therefore assumed that PD-1 plays an important role in the immune escape of tumor cells. In a “normal” immune response, this pathway prevents damage to collateral tissue during an inflammatory response. In NPC, both PD-L1 and PD-1 are highly expressed, and associations have been found between the extent of expression and the stage of disease, the interval to recurrent disease and overall survival. This implies a role for PD-1 in tumor growth and opens up new avenues as a potential target in treatment (22, 48- 49). Studies with anti-PD-1 in the treatment of melanomas showed promising results (50). Currently, two anti-PD-1 agents are being studied for use in patients with

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recurrent and/or metastatic NPC, i.e.; Nivolumab and Pembrolizumab. Preliminary results of Pembrolizumab showed tumor reduction in two-thirds of the patients. Taken together, a vast array of interesting and promising research is being done to attest these different approaches so as to improve the treatment of NPC (22, 49-52)

EBV and NPC

Undifferentiated and poorly differentiated NPC have a strong correlation with Epstein-Barr virus (EBV), whereas differentiated NPC is more related to smoking and drinking. In the high incidence NPC regions, the EBV genome and several actively expressed gene products are found in all tumor cells in up to 100% of the cases. In the low incidence regions this percentage is lower, although still more than half of the cases are related to EBV (15).

EBV is a ubiquitous human gamma herpes virus. In the general worldwide population, persistent infection of EBV ranges from 80 to 99%. Infection occurs via direct salivary contact. In developing countries, primary infection occurs in early infancy and is asymptomatic in most cases. In western countries primary infection is more often delayed until adolescence and then causes infectious mononucleosis in up to 25% of the cases (53).

In 1997, the World Health Organization (WHO) recognized EBV as a “Class 1 human carcinogenic virus” (54). Besides being associated with NPC, EBV is also associated with a variety of lymphoid (Burkitt’s lymphoma, Hodgkin’s Lymphoma, Lympho ephelioma-like carcinoma, extranodal NK/T- cell lymphoma, immunodeficiency- and transplant-associated B-cell lymphoma) and gastric cancers (55).

Research in NPC has focused on the usage of EBV-related biomarkers for risk screening, (early stage) diagnosis, predicting treatment outcome and early detection of recurrent disease. Patients with NPC have a strong immunoglobulin A (IgA) reactivity to EBV viral capsid antigens (VCA) and Epstein-Barr nuclear antigen 1 (EBNA1), and an overall broadened EBV-specific IgG/IgA antibody diversity (56-58). Furthermore, aberrant levels of EBV DNA can be detected in peripheral blood and in the nasopharyngeal region. All these markers have proven to be helpful for the diagnosis of primary NPC (59-61). Implementation of EBV-related biomarker testing and screening among risk populations may facilitate early-stage NPC detection, thereby improving timely initiation of treatment and overall outcome.

Since EBV is clonally present in all cells of EBV positive NPC, it is considered as a potential target for treatment (44). In NPC, EBV persists in a latent phase wherein only a few viral proteins and non-coding small RNAs are expressed. These are essential for EBV maintenance and tumor growth as they are non-immunogenic and contribute to NPC tumor immune escape (62-65). When EBV is triggered to enter the reproductive lytic phase, additional immunogenic proteins are expressed, which can provoke a stronger and more effective immune response (66). In addition, early in the lytic cycle, certain EBV-encoded kinases are newly expressed which can convert antiviral agents, like (val)ganciclovir, to become effective inhibitors of DNA synthesis, thus blocking (tumor) cell growth and virus replication. Several clinical trials have investigated the usage of EBV as target for treatment in NPC, but no breakthrough has been reached yet (44, 67-70). Personalized treatment using HLA-matched EBV-targeted T-cell therapies is showing some promise (71).

Indonesia

NPC is the most common malignancy in the head and neck region in Indonesia (3). The incidence is estimated at 6:100 000, which is probably an underestimation since many patients living in the rural areas may stay undiagnosed. In literature, 5-year overall survival for NPC is reported as 70 to 80%, or even higher (13, 15, 22). A previous study conducted in Yogyakarta, Indonesia, showed a 3-year overall survival of only 30% (72). Most of the studies with high survival rates are derived from clinics with advanced and readily available treatment facilities. As mentioned before, approximately 90% of the NPC patients are diagnosed in low- and middle-income countries, with less advanced equipment and limited capacity (3, 73). Therefore, actual NPC survival in low-and middle-income countries will be much lower than reported in literature and more likely to be in the range of that in Indonesia.

Indonesia has a population of 256 million. Eighty-seven per cent is Muslim, 7% is Protestant, 3% Catholic, 1.7% Hindu, 0.7% Buddhist and many other religions are found. The median age is 29.6 years, which is rather young compared to the Netherlands, where the median age is 42.3 years. In Indonesia 11.3% of the population lives in poverty. The health expenditure per year is 3.1% of the Gross Domestic Product, compared to 12.9% in the Netherlands (2012)(74). The health care physician density in Indonesia is 0.2 per 1000 people and there are 0.9 hospital

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beds per 1000 people, compared to 3.2 physicians and 4.7 hospital beds in the Netherlands (75).

Before 2014, the insurance system in Indonesia was complex and differed depending on the district. In general there were three types of insurances; 1) insurance for the very poor, if the patient fulfilled the set requirements, expenses were paid by the local or national government; 2) insurance for civil servants, which was an income based health insurance; and 3) patients who financed the health care themselves (private insurance or out of pocket payments) (76). Only half of the Indonesian population had an insurance in 2013 (77). The insurance for the poor was hampered by a lack of co-ordination between planning and implementation stages. It was, for instance, unclear how to one should go about getting this kind of insurance and people were poorly informed about their benefits. Here besides, only limited health care expenditures were covered and only so in certain health care centers (78). For the civil servants more health care services were covered, but also here problems were frequently encountered. Only when treatment could be paid out of the pocket, was health care readily delivered. Therefore, many Indonesians tried to collect money by any means possible when they or their close family needed healthcare. Consequently, it was not exceptional if a whole family or compound community went bankrupt when an expensive treatment, like radiotherapy, was needed.

Today, the Indonesian government's ambition is to improve the availability of and access to health care. Complete universal health care coverage is aimed for in 2018. In January 2014, the Healthcare and Social Security Agency, Badan Penyelenggara Jaminan Sosial Kesehatan (BPJS Kesehatan), was introduced to provide health care insurance for all citizens of Indonesia. All employers are required to register their employees; both the employer and employee contribute to a monthly premium. The self-employed or unemployed can also register and pay the premium themselves (79-80). To date, however, many people are still poorly informed about their benefits and to what they are entitled. Due to better health-care access, hospitals become overloaded and waiting lists are progressively getting longer. Such problems have now become clearly visible and more action from the government needs to be taken. Although there is much room for improvement, at least a start has been made.

Outline of the thesis

In the cases where nasopharyngeal cancer (NPC) persists or recurs survival is poor. When detected at an early stage, treatment is still feasible. **Part I** of this thesis is focused on early detection and improvement of the treatment of recurrent and persistent NPC. **Chapter 2** describes new diagnostics based on EBV that can be used in the follow-up of patients treated for primary NPC. It shows that the EBV-nasopharyngeal brush can detect local disease in the nasopharynx. **Chapter 3** contains a review on the treatment of locally persistent NPC. In **chapter 4**, photodynamic therapy as salvage treatment for local failures of NPC is analyzed in a prospective clinical trial. In **chapter 5**, a new treatment modality based on targeting the Epstein-Barr virus in NPC was used for recurrent and metastasized NPC.

Part II of the thesis is focused on Indonesia. Persistent and recurrent nasopharyngeal cancer is frequently seen in this region and therefore the survival rate of NPC is low. **Chapter 6** describes the problems during the primary treatment of NPC. It focuses on the reason why patients miss days during the treatment with radiotherapy. Missed days have to be rescheduled; therefore many patients have a prolonged overall treatment time. In **chapter 7** the effect of a prolonged treatment time on clinical outcome is presented. **Chapter 8** addresses the status of cancer care for young patients with nasopharyngeal carcinoma in Jakarta.

Finally, in **chapter 9**, the results obtained in this thesis are discussed. The aim of this thesis is to improve prognosis for people with persistent and recurrent NPC. Moreover, future perspectives, ongoing studies and suggestions for future research are discussed.

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